Fishery Data Series No. 08-22

Induction of Triploidy in Rainbow Trout (*Oncorhynchus mykiss*) using Hydrostatic Pressure

by

Diane P. Loopstra

and

Patricia A. Hansen

May 2008

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

FISHERY DATA SERIES NO. 08-22

INDUCTION OF TRIPLOIDY IN RAINBOW TROUT (*ONCHORYNCHUS MYKISS*) USING HYDROSTATIC PRESSURE

by
Diane P. Loopstra
Division of Sport Fish, Anchorage
and
Patricia A Hansen
Division of Sport Fish, Research and Technical Services, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1599

May 2008

Development and publication of this manuscript were partially financed by the Federal Aid in Sport fish Restoration Act (16 U.S.C.777-777K) under Projects F-32-13 and F-32-14, Job No. F-2-5.

The Division of Sport Fish Fishery Data Series was established in 1987 for the publication of technically oriented results for a single project or group of closely related projects. Since 2004, the Division of Commercial Fisheries has also used the Fishery Data Series. Fishery Data Series reports are intended for fishery and other technical professionals. Fishery Data Series reports are available through the Alaska State Library and on the Internet: http://www.sf.adfg.state.ak.us/statewide/divreports/html/intersearch.cfm This publication has undergone editorial and peer review.

Diane P. Loopstra
Alaska Department of Fish and Game, Division of Sport Fish,
333 Raspberry Road, Anchorage, Alaska 99518-1599, USA
and
Patricia A. Hansen
Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska 99518-1599, USA

This document should be cited as:
Loopstra, D., and P. A. Hansen. 2008. Induction of triploidy in rainbow trout (Oncorhynchus mykiss) using hydrostatic pressure. Alaska Department of Fish and Game, Fishery Data Series No. 08-22, Anchorage.

TABLE OF CONTENTS

Page

LIST OF TABLES ii
LIST OF APPENDICES iii
ABSTRACT 1
INTRODUCTION 1
METHODS 3
2004 3
Survival 4
Ploidy 4
2005 4
Survival 5
Ploidy 5
RESULTS 6
2004 6
Survival 6
Ploidy 7
2005 7
Survival 7
Ploidy 7
DISCUSSION 7
RECOMMENDATIONS 10
ACKNOWLEDGMENTS 10
REFERENCES CITED 10
APPENDIX A 11

LIST OF TABLES

Table **Page**

1. Results of studies using hydrostatic pressure shocking to induce triploidy in rainbow trout. 2
2. Treatments used in the hydrostatic pressure experiment in 2004 and 2005. 3
3. Survival and triploidy rates for each treatment group pressure shocked in 2004. 6
4. Average survival and triploidy rates for pressure-shocked treatments in 2004. 6
5. Flow cytometry results for each pressure-shocked treatment group in 2004. 8
6. Survival and triploidy rates for each treatment group pressure shocked in 2005. 8
7. Average survival and triploidy rates for pressure-shocked treatments in 2005. 9

LIST OF APPENDICES

Appendix **Page**

A1. Historic triploidy rates achieved by heat-shocked rainbow trout eggs at Fort Richardson Hatchery. 12

ABSTRACT

Hydrostatic pressure shocking was applied to fertilized rainbow trout (*Oncorhynchus mykiss*) eggs in 2004 and 2005 to induce triploidy. In 2004, eggs were pressure shocked for 5 minutes beginning at 300 Centigrade Temperature Minutes (CTMs) post fertilization with 9,000, 9,500, or 10,000 psi of pressure. Average survival rates from green egg to emergence for each treatment were greater than 70% of the control group. Triploidy rates ranged from 82.2% with 9,000 psi of pressure to 100% with 10,000 psi. In 2005, eggs were pressure shocked for 5 minutes beginning at 375 or 475 CTMs post fertilization with 9,000, 9,500, or 10,000 psi of pressure. Average triploidy rates ranged from 93.6 to 99.1%, and average survival rates to emergence ranged from 90.6 to 100%.

Key words: triploid, flow cytometry, rainbow trout, *Oncorhynchus mykiss*, survival, hydrostatic pressure, second polar body.

INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) produces triploid rainbow trout (*Oncorhynchus mykiss*) for stocking into lakes that have controlled or intermittent outlets, are flood prone, or are in a region where rainbow trout do not naturally occur. Triploid fish are incapable of reproduction, and therefore are unable to breed with native fish or establish a breeding population.

ADF&G currently produces triploid rainbow trout using a heat-shock treatment. The treatment is designed to prevent the extrusion of the second polar body during meiosis resulting in two sets of chromosomes being contributed by the female, and one set from the male. Triploidization rates of heat-shocked eggs are typically greater than 95%, but survival rates to emergence are as much as 18% lower than the survival rates of their diploid counterparts (ADF&G unpublished data).

Other treatments may also be used to produce triploid fish such as cold shock, hydrostatic pressure, and chemicals (Ihssen et al. 1990, Malison et al. 2001). In a laboratory study, Lincoln (1989) found that pressure-shocked rainbow trout eggs had higher egg and fry survival rates than heat-shocked eggs. At ADF&Gs Fort Richardson Hatchery (FRH), successful heat shocking requires maintaining a constant 26^{o}C water bath while masses of cool (8.5-10.0^{o}C) eggs are added. Eggs located near the center of the egg tray may not receive the same thermal shock treatment as eggs near the perimeter of the tray. This may affect triploidization and survival rates. Variation in egg size will also affect triploidization and egg survival rates (Teskeredzic et al. 1993). Eggs in a hydrostatic pressure treatment receive the same shock regardless of egg size or location within the pressure chamber.

Inducing successful triploidy with hydrostatic pressure depends largely on three factors: time of shock initiation (CTMs [Centigrade Temperature Minutes]), duration of the treatment (minutes), and the shock intensity (amount of pressure). Hydrostatic pressure shocking has been successful in inducing triploidy in rainbow trout (Table 1) (Chourrout 1984; Hamor et al. 1996; Lincoln 1989; Lou and Purdom 1984; Wickwire 2000; Yesaki et al. 1996).

The amount of pressure, shock duration, and timing of shock initiation used to achieve triploid rainbow trout differ between studies (Chourrout 1984; Hamor et al. 1996; Lincoln 1989; Lou and Purdom 1984; Wickwire 2000; Yesaki et al. 1996). Triploidy rates of 100% have been achieved using pressures ranging from 7,000 to 10,400 psi, with 4 or 5 minute shocks initiated at 300 to 400 CTMs post fertilization.

Table 1.-Results of studies using hydrostatic pressure shocking to induce triploidy in rainbow trout.

Researcher	Centigrade Temperature Minutes	Pressure (psi)	Duration (minutes)	Percent triploid
Chourrout (1984)	376	7,000	4	100
Hamor et al. (1996)	306	10,000	4	100
Hamor et al. (1996)	202	10,000	4	80
Lincoln (1989)	400–500	9,000	3-8	92 to 99
Lou and Purdom (1984)	40	8,000	10	80 to 90
Wickwire (2000)	300	9,150; 9,700; 10,400	5	100
Yesaki et al. (1996)	250	8,500	5	5
Yesaki et al. (1996)	400	8,500	5	68
Yesaki et al. (1996)	250	9,500	5	80
Yesaki et al. (1996)	400	9,500	5	100

Chourrout (1984) reported 100% triploidy rates using 7,000 psi of pressure for 4 minutes beginning at approximately 376 CTMs post fertilization, while Yesaki et al. (1996) reported only 68% triploidy rates for eggs shocked at 400 CTMs with 8,500 psi of pressure. Lou and Purdom (1984) achieved 80–90% triploidy rates by shocking eggs at 8,000 psi for 10 minutes beginning 40 minutes post fertilization. Lincoln (1989) reported that 9,000 psi was the lowest pressure that consistently produced high triploidy rates. He also reported highest triploidy and survival rates among egg groups shocked at 400 or 500 CTMs post fertilization, yet Wickwire (2000) reported 100% triploidy rates for eggs shocked at 300 CTMs post fertilization with 9,150 to 10,400 psi of pressure.

The purpose of this study was to find a hydrostatic pressure shocking protocol for inducing triploidy in rainbow trout that could improve survival rates, and maintain or improve triploidy rates over what is currently achieved by heat shocking at FRH. The specific objectives were to:

1. Estimate the mean survival rate from fertilization to the eyed-egg stage for each treatment group (Table 2).

For those treatment groups with an eyed-egg survival rate greater than or equal to 70% of the control:

2. Determine which treatment produced the highest rate of triploidization.

A goal of this study in 2005 was to improve survival rates while maintaining the high triploidy rates achieved in 2004. Because heat-shocked triploid rainbow trout develop spinal deformities (up to about 10%), pressure-shocked rainbow trout were also examined for deformities.

Table 2.-Treatments used in the hydrostatic pressure experiment in 2004 and 2005.

	Treatment	Time from fertilization to shock (min)	Centigrade Temperature Minutes (CTMs)	Hydrostatic pressure (psi)	Pressure shocking duration (minutes)
2004					
	1	36	300	9,000	5
	2	36	300	9,500	5
	3	36	300	10,000	5
2005					
	1	47	375	9,000	5
	2	47	375	9,500	5
	3	47	375	10,000	5
	4	59	475	9,000	5
	5	59	475	9,500	5
	6	59	475	10,000	5

Note: The time from fertilization to shock initiation is based on water bath temperatures of 8.3°C in 2004 and 8.1°C in 2005.

METHODS

2004

Eggs and milt were collected from captive Swanson River rainbow trout broodstock on 15 April 2004. Approximately 15 ml of eggs collected from each of eight females were combined in a Ziploc® bag and stored in a cooler on ice. Milt was collected from four males (XY genotype) and stored in separate vials in a cooler on ice. Before fertilization, a sample of milt from each vial was activated with a 120 mM solution of NaCl and examined with a microscope to verify the presence of motile sperm.

Approximately half of the egg mixture was transferred to a fertilization container. These eggs were used for one replicate. An equal volume of milt from each of the four males was added, and sperm was activated with the saline solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 8.3°C water 1 minute post fertilization. The fertilized eggs in these treatments produced both male and female offspring.

For each replicate, an estimated 15 ml of fertilized eggs were placed into each of four egg tubes (each egg tube contained approximately 200 eggs), one for each treatment and a control group. The egg tubes were submerged in an 8.3°C ±0.1°C water bath where the eggs water hardened for nearly 300 CTMs before pressurization.

Eggs were pressure shocked in one of three 24 oz stainless steel chambers fitted with a double O-ring brass piston. Approximately 5 minutes before the 300 CTM shock initiation time, the chambers were filled with 8.3°C water and egg tubes for the first replicate treatment groups were gently lowered into each chamber. The pistons were set in place, and all air and excess water were expelled via a side relief valve before sealing the chambers. A 12-ton shop press with a 12-ton bottle jack was used to achieve 9,000 psi within one pressure chamber, a 15-ton shop press

was used with a 20-ton jack to achieve 9,500 psi within a second pressure chamber, and a 25-ton jack was used with a 25-ton press to achieve 10,000 psi in a third pressure chamber.

The pressure-shock treatments began when the desired pressure was achieved within each chamber. After 5 minutes, each chamber was rapidly depressurized, and the egg tubes were transferred back to the 8.3°C water bath. At 45 minutes (approximately 373 CTMs) post fertilization the egg tube containing the control group was placed in a non-pressurized chamber. The control eggs remained in the non-pressurized chamber for 5 minutes and were then returned to the 8.3°C water bath. At 60 minutes (approximately 498 CTMs) post fertilization all eggs were transferred from the egg tubes to individual incubation containers and disinfected with an iodophor solution (1:100 concentration) for 15 minutes.

Eggs for the second replicate were transferred to the fertilization container and fertilized as described for the first replicate. The two replicates were incubated in separate egg trays with each tray containing four incubation containers.

Survival

At the eyed-egg stage, dead eggs were removed and enumerated. The remaining eggs in each incubation container were physically shocked by pouring them into a bucket of water from a height of approximately 1 foot to turn dead eggs white. After 24 hours, the remaining dead eggs were removed and enumerated. Live eggs were returned to their incubation container. Dead eggs, dead alevins, live and dead fry were enumerated at emergence. The number of live emergent fry with obvious deformities was also recorded. Simple binomial proportions were used to calculate the survival rate for each group, and treatment survival rates were estimated by averaging replicates.

Ploidy

Flow cytometry was used to analyze blood and slime cells for ploidy (Thorgaard et al. 1982). The cells were preserved in a solution containing the DNA binding fluorescent dye 4',6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) with 10% dimethyl sulfoxide (DMSO) (Thornthwaite et al. 1980). Samples were analyzed from at least 45 fish in each group that achieved the minimum 70% survival rate to eyed-egg stage criteria of the control group survival rate. Samples from diploid fish were also collected to use as controls. If the triploidization rate of the first 10 samples of a group was less than 80%, then no further samples from that group were tested. Simple binomial proportions were used to calculate the triploidization rate for each group, and triploidization rates were estimated by averaging across replicates.

2005

Eggs and milt were collected from captive Swanson River rainbow trout broodstock on 21 April 2005. Approximately 20 ml of eggs collected from each of 11 females were combined in a Ziploc® bag, and stored in a cooler on ice. Testes collected from seven masculinized (XX genotype) males were macerated to release the milt, and stored in individual Whirl-pak® bags in a cooler on ice. Sperm motility, in terms of percent motile and duration, was visually checked for each male by using a microscope to examine a drop of milt added to a 120 mM solution of NaCl. Milt from four males was selected for fertilization.

Approximately 70 ml of the egg mixture was transferred to a fertilization container. These eggs were used for one replicate. An equal volume of milt from each of the four males was added,

and sperm were activated with the saline solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm were rinsed away with 8.1°C water 2 minutes post fertilization. The fertilized eggs in these treatments produced all female offspring.

For each replicate, approximately 10 ml of fertilized eggs were placed into each of seven egg tubes (each egg tube contained approximately 100 eggs): one for each treatment and a control group. The eggs were submerged in an 8.1°C ±0.2°C water bath. Using 8.1°C water, the 375 and 475 CTM egg groups were water hardened for approximately 47 minutes and 59 minutes, respectively, before they were pressure shocked.

Eggs were pressure shocked in one of three 24 oz stainless steel chambers fitted with a double O-ring brass piston. Approximately 5 minutes before the 375 CTM shock initiation time, each chamber was filled with 8.1°C water, and egg tubes for the first replicate were gently lowered into each chamber. Each chamber was sealed and pressurized as described for the 2004 treatment groups. This procedure was repeated for the 475 CTM treatment groups.

The pressure-shock treatments began when the desired pressure was achieved within each chamber. After 5 minutes, each chamber was rapidly depressurized and the egg tubes were transferred back to the water bath. At 70 minutes (approximately 567 CTMs) post fertilization, the egg tube containing the control group was placed in a non-pressurized chamber. The control eggs remained in the non-pressurized chamber for 5 minutes before being returned to the water bath. At 84 minutes (approximately 680 CTMs) post fertilization, all eggs were transferred to an iodophor solution (1:100 concentration) for a 15-minute disinfection. Disinfected eggs were then transferred to an incubation container within an egg tray.

The fertilization and shocking processes were repeated for the second replicate using milt from the same four males and the same egg mix used in replicate one.

A water temperature fluctuation altered the shock initiation time (CTMs) for the 475 CTM treatments in the first replicate by an unknown number of CTMs. These eggs were discarded and replaced with additional fertilized eggs.

Survival

At the eyed-egg stage, dead eggs were removed and enumerated. Because the eggs were on the verge of hatching, they were not physically shocked or enumerated as described for the 2004 treatment groups. At emergence, any remaining dead eggs, dead alevins, live and dead fry, and fry with obvious deformities were enumerated. Simple binomial proportions were used to calculate the survival rate for each group, and treatment survival rates were estimated by averaging across replicates.

Ploidy

Blood and slime samples were collected and preserved as described for the 2004 treatments. For each group, the ploidy of at least five alevins was checked using flow cytometry. Simple binomial proportions were used to calculate the triploidization rate for each group, and triploidization rates were estimated by averaging across replicates. Survival and triploidy rates were ranked to determine the most effective treatment.

RESULTS

2004

A needle valve failure prevented the pressure chamber for the first replicate 9,000-psi treatment group from holding at the desired pressure. Although additional pressure was applied throughout the treatment, the pressure in the chamber varied between 8,800 and 9,200 psi. The needle valve leak worsened during the second replicate, and the treatment was discontinued after failing to achieve 9,000 psi of pressure. Therefore, there is only one replicate for the 9,000-psi treatment group.

Survival

Survival rates from green egg to emergence for the individual 9,000, 9,500, and 10,000 psi treatment groups ranged from 63.0 to 88.8% (Table 3). Average survival rates from green egg to emergence by treatment ranged from 74.2% for the 10,000 psi group to 86.4% for the 9,000 psi group (Table 4). The percentage of fish with deformities within individual treatment groups ranged from 0.0 to 7.9% (Table 3). The second replicate of pressure-shocked fish had a higher percentage of deformities (7.4–7.9%) than the first replicate (0.0–1.3%).

Table 3.-Survival and triploidy rates for each treatment group pressure shocked in 2004.

			Shock	Green egg to eyed egg		Eyed egg to emergence		Green egg to emergence			
			duration		Survival relative		Survival relative		Survival relative	Percent	Percent
CTMs[a]	Pressure	Replicate	(min)	Survival	to control	Survival	to control	Survival	to control	triploid	deformities
control group		1		96.3%		98.6%		95.0%			1.4%
300	9,000	1	5	84.2%	87.4%	97.5%	98.9%	82.1%	86.4%	82.2%	1.3%
300	9,500	1	5	86.3%	89.5%	97.8%	99.2%	84.4%	88.8%	91.8%	0.0%
300	10,000	1	5	83.1%	86.2%	83.8%	85.0%	69.7%	73.3%	100.0%	0.0%
control group		2		91.8%		99.0%		90.9%			1.0%
300	9,000	2[b]	5								
300	9,500	2	5	66.2%	72.1%	86.5%	87.4%	57.3%	63.0%	100.0%	7.4%
300	10,000	2	5	75.8%	82.6%	89.9%	90.8%	68.2%	75.0%	100.0%	7.9%

[a] Centigrade Temperature Minutes

[b] Replicate not completed because of equipment failure.

Table 4.-Average survival and triploidy rates for pressure-shocked treatments in 2004.

		Shock	Green egg to eyed egg		Eyed egg to emergence		Green egg to emergence		
		duration		Survival relative		Survival relative		Survival relative	Percent
CTMs[a]	Pressure (psi)	(min)	Survival	to control	Survival	to control	Survival	to control	triploid
control group			94.1%		98.8%		92.9%		
300	9,000[b]	5	84.2%	87.4%	97.5%	98.9%	82.1%	86.4%	82.2%
300	9,500	5	76.2%	81.0%	92.2%	93.3%	70.8%	76.2%	95.9%
300	10,000	5	79.4%	84.4%	86.9%	87.9%	68.9%	74.2%	100.0%

[a] Centigrade Temperature Minutes.

[b] 9,000 psi treatment includes data for one replicate only.

Ploidy

All treatments achieved the minimum 70% average survival rate of the control groups necessary to be tested for ploidy. Three individual treatment groups (both 10,000 psi replicates and one 9,500 psi replicate) achieved 100% triploidy rates (Table 3). The 10,000 psi treatment produced the highest average triploidy rate (100%), but the lowest (74.2%) average survival rate from green egg to emergence. The 9,000 psi treatment produced the lowest average triploidy rate (82.2%), but the highest (86.4%) average survival rate (Table 4).

Three fish sampled from the first replicate 9,000 psi and 9,500 psi treatment groups contained cells with DNA amounts that peaked between the known diploid and triploid peaks on the flow cytometry histogram. The 9,500 psi treatment group contained one diploid fish (2.0%), and the 9,000 psi treatment group contained five diploids (11.1%) (Table 5).

2005

Survival

Survival rates relative to the control groups from green egg to emergence for individual treatment groups ranged from 87.5 (9,000 psi 375 CTMs) to 106.3% (9,500 psi 375 CTMs) (Table 6). Average survival rates relative to the control groups from green egg to emergence for each treatment type ranged from 90.6 (9,000 psi 375 CTMs) to 100% (9,500 psi 375 CTMs) (Table 7). The percentage of fish with deformities within individual treatment groups ranged from 0.0 to 5.8% (Table 6).

Ploidy

All treatments achieved the minimum 70% average survival rate of the control groups necessary to be tested for ploidy. A 100% triploidy rate was achieved by four individual treatment groups (Table 6), but none of the treatment groups achieved a 100% average triploidy rate (Table 7). The lowest triploidy rates (90.9 and 96.4%) were achieved by both 475 CTM treatments shocked with 9,000 psi of pressure (Table 6). All other groups achieved 98.2 to 100% triploidy rates. No fish with cells containing a DNA amount between that of diploid and triploid fish were found.

DISCUSSION

The 300 CTM treatments were based on the success of Wickwire (2000). Although Wickwire reported 100% triploidy rates for treatments shocked at 300 CTMs with 9,150, 9,700, or 10,400 psi of pressure, only the 10,000 psi treatment group in 2004 achieved 100% triploidy in our study. However, the low (74.2%) average survival rate achieved by this treatment group is not an improvement over the survival rates currently achieved by heat-shocked production lots of rainbow trout eggs produced at FRH. Fungal growth may have contributed to the poor survival in the 300 CTMs treatment groups. A leaking pressure valve may have contributed to the low (82.2%) triploidy rate achieved by the 9,000 psi treatment group in 2004.

An inverse relationship between survival and triploid rates relative to increasing shocking pressure described by Hamor et al (1996) was observed in the 300 CTM treatments, but not for treatments shocked at 375 or 475 CTMs. Groups shocked with 9,500 psi of pressure had the best survival rates of the 375 (100%) and 475 (97.7%) CTM treatment groups, and acceptable (99.1%) triploidy rates.

Survival and triploidy rates support the finding by Hamor et al (1996) that shock initiation time (CTMs) is the most important variable when using pressure-shock treatments to induce triploidy in rainbow trout. The 375 and 475 CTM shock initiation times yielded more successful

Table 5.-Flow cytometry results for each pressure-shocked treatment group in 2004.

		Ploidy		
Pressure (psi)	Replicate	Percent triploid (3N)	Percent diploid (2N)	Percent between 2N and 3N
9,000	1	82.2%	11.1%	6.7%
9,500	1	91.8%	2.0%	6.1%
10,000	1	100.0%	0%	0%
9,000	2[a]			
9,500	2	100.0%	0%	0%
10,000	2	100.0%	0%	0%

[a] Replicate not completed because of equipment failure.

Table 6.-Survival and triploidy rates for each treatment group pressure shocked in 2005.

			Shock	Green egg to emergence			
CTMs[a]	Pressure (psi)	Replicate	duration (min)	Survival	Survival relative to control	Percent triploid	Percent deformed
control group		1		91.5%			0.9%
375	9,000	1	5	80.0%	87.5%	98.2%	2.4%
375	9,500	1	5	86.0%	94.1%	98.2%	2.7%
375	10,000	1	5	89.7%	98.1%	98.2%	2.3%
control group		1[b]		87.6%			0.0%
475	9,000	1[b]	5	80.8%	92.3%	96.4%	2.6%
475	9,500	1[b]	5	85.5%	97.5%	98.2%	3.5%
475	10,000	1[b]	5	79.9%	91.2%	100.0%	1.7%
control group		2		86.3%			0.8%
375	9,000	2	5	81.1%	93.9%	100.0%	5.8%
375	9,500	2	5	91.7%	106.3%	100.0%	2.5%
375	10,000	2	5	84.2%	97.5%	98.2%	3.8%
475	9,000	2	5	80.1%	92.8%	90.9%	4.0%
475	9,500	2	5	80.9%	93.7%	100.0%	3.5%
475	10,000	2	5	78.3%	90.7%	98.2%	0.0%

[a] Centigrade Temperature Minutes.

[b] Replacement fertilized eggs (from the same females and males) were used for the first replicate 475 CTM treatments and another control group because of a water temperature fluctuation during the treatment.

Table 7.-Average survival and triploidy rates for pressure-shocked treatments in 2005.

CTMs[a]	Pressure (psi)	Shock duration (min)	Green egg to emergence Survival	Survival relative to the control	Percent triploid
control group			88.9%		
375	9,000	5	80.5%	90.6%	99.1%
375	9,500	5	88.9%	100.0%	99.1%
375	10,000	5	87.0%	97.8%	98.2%
control group			87.0%		
475	9,000	5	80.5%	92.5%	93.6%
475	9,500	5	83.2%	95.6%	99.1%
475	10,000	5	79.1%	90.9%	99.1%

[a] Centigrade Temperature Minutes.

combinations of survival and triploidy rates than the 300 CTM shock initiation times. These findings also support those of Lincoln (1989) who reported higher triploid and survival rates for treatments shocked at 400 and 500 CTMs than those shocked at 300 CTMs.

The presence of deformed fry in 14 of 17 pressure-shocked treatment groups suggests that pressure shocking does not eliminate the occurrence of spinal deformities in triploid populations. Three of the four control groups also contained fish with spinal deformities, which suggests some deformities may be inherited. There does not appear to be a relationship between pressure or shock initiation time and incidence of deformity.

Flow cytometry for one of the 300 CTM 9,000 psi and 9,500 psi treatment groups revealed samples with histogram peaks located between the known peaks for diploids and triploids; thus indicating the DNA amount in the nucleus is greater than that of the diploid controls, but less than that in triploid samples. These two treatment groups also contained diploids. Chourrout (1984) suggested these embryo types (called aneuploids) are the result of a low intensity shock, but samples from other 9,000 and 9,500 psi treatment groups did not contain aneuploids. Embryos that died before hatching were not retained (included in the samples), so it is possible that aneuploids were present but not identified. Both samples containing aneuploids were shocked at 300 CTMs suggesting the stage of cell development at shock initiation may be a factor.

The best combination of survival and triploidy rates was achieved with 9,500 psi of pressure for 5 minutes at 375 CTMs post fertilization. The 99.1% triploidy rate is comparable to or better than the historic triploidy rates achieved by heat-shocked production eggs at FRH (Appendix A1), and the survival rate (at the experimental level) is greater than the rates for heat-shocked eggs (at the production level). If these survival and triploidy rates can be maintained at a production level, pressure shocking rainbow trout eggs to induce triploidy would be an improvement over the heat-shock method currently used at FRH.

RECOMMENDATIONS

1. Pressure shock rainbow trout eggs with 9,500 psi of pressure at 375 CTMs post fertilization for 5 minutes to induce triploidy.
2. Because 100% triploidy rates are not consistently achieved with pressure-shocked or heat-shocked rainbow trout, make triploid populations all female.
3. Compare survival, triploidy, and deformity rates of Swanson River broodstock rainbow trout that were pressure shocked to induce triploidy to those that were heat shocked.
4. Spinal deformities in heat-shocked rainbow trout are often not evident until the fish weighs approximately 10 g. Pressure-shocked and heat-shocked rainbow trout should be reared to at least 10 g to compare the rate of spinal deformities in the populations.

ACKNOWLEDGMENTS

We would like to thank the staff at Fort Richardson Hatchery for providing the rainbow trout gametes, incubation space, and advice throughout this study.

REFERENCES CITED

Chourrout, D. 1984. Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous diploid gynogenetics. Aquaculture 36:111-126.

Hamor, T., R. Beck, and J. Stewart. 1996. Alteration of ploidy in rainbow trout with heat and hydrostatic pressure. Proceedings of the 47th Annual Northwest Fish Culture Conference, 174-186.

Ihssen, P. E., L. R. McKay, I. McMillan, and R. B. Phillips. 1990. Ploidy manipulation and gynogenesis in fishes: cytogenetic and fisheries applications. Transactions of the American Fisheries Society 119:698-717.

Lincoln, D. 1989. Triploid induction in rainbow trout using hydrostatic pressure. Trout News 8:8-10.

Lou, Y. D., and C. E. Purdom. 1984. Polyploidy induced by hydrostatic pressure in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology 25:345-351.

Malison, J. A., J. A. Held, L. S. Weil, T. B. Kayes, and G. H. Thorgaard. 2001. Manipulation of ploidy in walleyes by heat shock and hydrostatic pressure shock. North American Journal of Aquaculture 63:17-24.

Teskeredzic, E., E. M. Donaldson, Z. Teskeredzic, I. I. Solar, and E. M. Lean. 1993. Comparison of hydrostatic pressure and thermal shocks to induce triploidy in coho salmon (*Oncorhynchus kisutch*). Aquaculture 117:47-55.

Thorgaard, G. H., P. S. Rabinovich, M. W. Shen, G. A. E. Gall, J. Propp, and F. M. Utter. 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture 29:305-310.

Thornthwaite, J. T., E. V. Sugarbaker, and W. J. Temple. 1980. Preparation of tissues for DNA flow cytometric analysis. Cytometry 1:229-237.

Wickwire, R. 2000. Salmonid triploiding experiment California Department of Fish and Game. Found at www.kokaneepower.org/articles/triploid.pdf (accessed on February 1, 2007).

Yesaki, T. Y., K. W. Scheer, and D. L. Greiner. 1996. Production-scale pressure shocking of rainbow trout (*Oncorhynchus mykiss*) in British Columbia. Proceedings of the 47th Annual Northwest Fish Culture Conference, 170:173.

APPENDIX A

Appendix A1.-Historic triploidy rates achieved by heat-shocked rainbow trout eggs at Fort Richardson Hatchery.

Brood year[a]	Sample size	# triploid	% triploid
1996	150	149	99.3%
1997	150	147	98.0%
1999	155	154	99.4%
2000	150	149	99.3%
2001	150	150	100.0%
2002	150	144	96.0%
2003	228	223	97.8%
2004	313	305	97.4%
2005	162	157	96.9%
Average of all years:			98.2%

[a] Information for brood year 1998 is missing.